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I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

I also certify that the attached copy of the request for grant of a Patent (Form 1/77) bears an amendment, effected by this office, following a request by the applicant and agreed to by the Comptroller-General.

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Signed P. Mahoney

Dated 20 June 2000

Patents Form 1/77

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The Patent Office

1/77

Request for grant of a patent

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1. Your reference

2. Patent ap... **9904777.1**
(The Patent Office will ...)

03MAR99 E429555-1 C49764
P01/7700 0.00 - 9904777.1

-2 MAR 1999

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

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Patents ADP number *(if you know it)* 761307800 1

If the applicant is a corporate body, give the country/state of its incorporation

4. Title of the invention

NOVEL METHOD
AN ASTHMA THERAPY THAT ACT ON EOSINOPHILS AND/or
T-Lymphocytes

5. Name of your agent *(if you have one)*

Correspondance To 761308600 1

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

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Patents ADP number *(if you know it)*

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*
	Kath Sarigisson 70 Stubbard Avenue Baldertan	

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application Newark Notts NG24 3EG

Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

	Continuation sheets of this form	/
43	Description	page 4-44
1	Claim(s)	THREE (3)
2	Abstract	page 15 / page 38
2.	Drawing(s)	4 Microscopical photo-, 16 tables, 18 figures

10. If you are also filing any of the following, state how many against each item.

Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

11. I/We request the grant of a patent on the basis of this application.

Signature N. Nassim Date 2/4/1999

12. Name and daytime telephone number of person to contact in the United Kingdom

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

a) If you need help to fill in this form or you have any questions, please contact the Patent Office on 0645 500505.

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e) Once you have filled in the form you must remember to sign and date it.

f) For details of the fee and ways to pay please contact the Patent Office.

THE DRUG TO BE PATENTED:

(1) **COMMERCIAL NAME:** INMUNOFERON

Mentioned in the text as drug (N)

ACTIVE INGREDIENTS: GLICOFOSFOPEPTICAL (3905-W)
(Fosfoglicopeptical)

sour· MARTINDALE THE EXTRA PHARMA
PRODUCING COMPANY:
LABORATORIOS ANDROMACO S.A.,
AZCONA, 31 - 28028, MAPRID, SPAIN.
THE NEW TRADE NAME:

/

A NEW DRUG APPPLICATION (NDA) FOR THE ABOVE MENTIONED DRUG, IN THE TREATMENT OF DISEASES MEDIATED BY TYPE 1 HYPER SENSITIVITY REACTION, MAINLY ASTHMA

(2) A NOVEL B.R.M. (BIOLOGIC RESPONSE MODIFIER)
NIGELLA SATIVUM NATURAL HERB
ORALLY ADMINSTERED
PURE POWDER IN CPSULES OR SUSPENSIOI
200 - 800 mg / dose
THREE TIMES / day
INDICATIONS : PAGE 2

(3) OTHER BIOLOGIC RESPONSE MODIFIERS (IMMUNOMODULATORS) IN TYPE I HYPERSENSITIVITY REACTON ON SHORT TERM BASIS

Indications for the therapeutic use of Substance (A):

NIGELLA SATIVUM NATURAL HERB

For the treatment of diseases associated with a defect in cell mediated immunity

I – Diseases caused by type I hyper sensitivity, such as:

1. Asthma.
2. Laryngeal oedema (Angioneurotic).
3. Allergic Rhinitis.
4. Allergic Conjunctivitis.
5. A topic dermatitis.

II- Acute & recurrent UTI.

III- Pelvic inflammatory diseases.

IV- Viral respiratory tract infections (flue & influenza).

V- Other viral infections.

VI- Cancer therapy.

2A ~~2B~~ FOLLOWS ON LINE ARTICLES AT THE END

Advantages:

The following advantages had been noticed, with drug (N) and substance (A) therapy, for **ASTHMA** during clinical trial:

I- **Enables the patient to live a more rather normal life** as it is capable of.
Meeting most of the goals of asthma therapy mentioned earlier.

II- **Short course** of therapy of **30 capsules,** or what is equivalent in-total during the course.

III- The course of treatment can be repeated as needed at intervals.

IV- **Enabling the patient to reduce** other conventional anti-asthma therapy because they don't need them as they are symptom-free.

V- **Tapering corticosteroids** and weaning from it.

VI- **Patient compliance is very good,** because this treatment is capable of achieving the confidence of the chronically ill patient with some psychological element from the disease. ie, suffering continuous use of conventional therapy with partial relief of his symptoms.

VII- **Side effects are few,** mild epigastric pain some times occurs with a large dose,
No other side effects were noticed during treatment.
Complete blood picture shows **no** abnormalities.
Blood urea and creatinin were normal during treatment.
Liver function (total serum bilirubin, SGPT, alkaline.
Phosphatase) were normal.
General urine analysis is normal.

Substance (A) had been used in humans for quite some time with out a noticeable toxic or undesirable effects.

Drug (N) is already present in the market for human use.

VIII- The results of this therapeutic approach lead to the formation of theory in the immunopathogeneses of type I hyper sensitivity namely **a selective switching-off of the eosinophilic inflamtion by stimulating the function of regulatory T Lymphocytes**

ASTHMA

Definition:

Asthma is a chronic inflammatory disorder of the airways. This causes recurrent episodes of wheezing, breathlessness, chest tightness and cough particularly at night and /or in the early morning. These symptoms are usually associated with wide spread but variable airflow limitation, and is at least partly reversible either spontaneously or with treatment.[1]

Incidence

There are between 100 and 150 million people in the world, including many children, who do not take breathing for granted. For them it can be a life and death struggle against recurrent attacks of breathlessness and wheezing caused by asthma. Each year, arround 180 000 of these sufferers lose the battle and die of the disease.[2]

PATHOGENESIS OF ASTHMA

An understanding of the pathogenesis and pathophysiology of this disorder provides the opportunity **for improved therapeutic intervention.**[3]

The pathogenesis of asthma is **complex and not fully understood**, it involves a number of cells, mediators, nerves and vascular leakage that can be activated by several different mechanisms, of which exposure to allergens is the most important.[4] (Fig. 1)

Numerous hypotheses have been put forth to explain the clinical syndrome of asthma. Recently, an appreciation of airway inflammation in asthma has led to reevaluation of all the concepts in the pathogenesis. Various in vivo and in vitro experiments suggest that, in susceptible host, **a T-cell mediated immune response to inhaled antigen occur.**[3]

The pathological features of asthma have also been investigated in patients by means of sputum examinations, bronchoatveolar Lavage (BAL) and endobronchial biopsy, all of inflamatory cells. Eosinophils in the sputum have been suggested as a marker of asthma.[3]

In patients dying of fatal exacerbation of asthma, microscopical examinations of the lungs revealk many changes, the most readily apparent and consistent features of the inflammatory cell infiltrate, particularly eosinophils, Lymphocytes and other inflammatory cells.

Endobronchial biopsy specimens reveal inflammatory cell influx similar to that found in postmortem speciems of airways, **even when diseases are relatively quiscent.**[3]

Immediate hypersensitivity reaction refers to a collection of signs and symptoms comprising respiratory, cutaneous, cardiovascular, gastrointestinal and systemic responses to a variety of pharmacologically active proinflammatory substances called **mediators**.[3b]

These reactions require the concerted interactions of sensitizing antibodies, specific target cells and mediators.[3b] **Any one of these can be targeted as a therapeutic weapon.**

Antibodies responsible for immediate hypersensitivity reaction.
First where described by Prausnitz and Kustner in 1921, 40 years later it was purified and called **IgE**. It is a cytophilic antibody that binds specific surface receptors of mast cells. Cross- linking of two IgE antibody molecules results in mast cell degranulation. IgE is under complex regulatory control, genetic influences are important.[3]

In humans type 1 reaction is mediated by IgE antibodies. **The differentiation of IgE secreting B cells is highly dependant on the induction of CD4+ helper T cells of TH2 type.** The first step in the synthesis of IgE is the presentation of the antigen (allergene) to precursors of TH2 cells by antigen presenting **dendritic cells**. The newly minted TH2 cell produce a cluster of **cytokines**, including IL-3, IL-4, IL-5 and GM-CSF, of these, IL-4 is absolutely essential for turning on the IgE-producing B cell.[5]

Humans T helper (CD4+) cells can be divided into subsets, TH1 and TH2, based on the profile of cytokines they produce. This is important in selecting effector function.(Fig.2)

TH1 cell: involved in cytotoxic, inflammatory and delayed type hypersensitivity. Interferon-gamma (IFN) has modulatory effect on immune function important in transfer of antigenic information to T lymphcytes.[7]
Reduced IFN at birth is noticined in babies born to families with a history of atopic allergic diseases.[7]

TH2 cell: encourages production of IgE & regulation of allergic response. IL 5 released by TH2 cells are chemotactic for eosinophils & enhance their release of mediators & cytokine.[6]

Cytokines from TH1 cells inhibit the action of TH2 cells & vice versa.
The cytokine environment determines which T-cell subset is produced from TH0 cells, and therefore which pathway B cells will take. (Fig.3)

T cells deficiency is associated with atopy: there is substantial evidence for a role for T-cells in both the development & suppression of IgE responses.
This led to the earlier suggestions that a **defect in T-cells, and in particular suppressor T-cells could be involved in the etiology of atopy** [6].

Human mast cells have been demonstrated to synthesize and release a variety of cytokines and growth factors, postulated in eosinophil growth, maintenance, recruitment, and activation; in IgE synthesis; and in fibrosis.

The pattern of mast cell cytokine synthesis is similar to TH2 helper T Lymphocytes and may be important contributor to allergic inflammation, such as that noted in asthma. *

Eosinophiles: these are non- dividing granular cells which arise principally in the bone marrow. Eosinophil differentiation, like that of all leukocytes is influenced by cytokines[7], they constitute up to 5% of white blood cells in healthy individuals and appear to be used selectively for fighting parasitic infection, they participate in hypersensitivity (allergic) reactions [4]. In allergic inflammatory disorders chaemokines such as Macrophage chaemoattractant protein (MCP) and eotaxin from macrophages, activated leukocytes & endothelial cells bind to receptors in the eosinophiles, inducing cell migration and activation [4].

Extravasation of leucocytes involve :

1. In the lumen arigration , rolling, adhesion.
2. Transmigration across the endothelium (diapedesis)
3. Migration in interstitial tissues toward a chemotactic stimulus.(robbins P 56:

Eosinophil Chaemotactic Factors:

Various factors are released from different cells that has the ability to attract eosinophils to migrate to the tissue, these include:

- Mast cell degranulation & basophils release an Eosinophil Chemotactic Factor (ECF) , **A low – molecular weight peptide (300-500 daltons)** . Also a preformed , immunologically releaseable , **chemotactic factor (1500-3000 daltons)** whith specificity for eosinophils .[7]

- Similar factors have been identified in the circulation of patients after **experimental induction of physical urticaria** or antigen provoked bronchospasm.

- Other ECF can be generated by **the lipoxygenase – dependant pathway of arachidonic acid metabolism .**[7]
- **Platlet Activating Factor (PAF)** is also a potent leukoattractant, particularly for eosinophils.

- **Rantens**

MANAGEMENT OF ASTHMA

The principles of management of asthma are based closely on the guidelines for the management of asthma produced by the British Thoracic society and also the International Consensus Report on the diagnosis and management of asthma (Fig.)

Expert panel report 2 from the National Asthma Education and prevention program of the National Heart, Lung and Blood Institute recommended a step wise approach to therapy (tab) (center 99).

Management consists of:

A) Environmental control and antigen avoidance.

B) Immunotherapy or hyposensitization.

C) Pharmacological agents, that can be divided into:

1)**Quick relief mediation**: taken to promote prompt reversal of acute airway obstruction and relief of accompanying symptoms by direct relaxation of smooth muscle, table (ref. current).

2)**Long term control** of persistent asthma, also referred to as maintenance, controller or preventive medications act primarily to attenuate airway inflammation. table (ref. current 99).

Table 9–5. Selected drugs for obstructive airway diseases.[1]

Drug	Important Formulations	Usual Adult Dosage (Stable Patient)	Comments
BRONCHODILATORS			
Sympathomimetics			
Albuterol (Proventil, Ventolin, Volmax)[2]	Metered-dose inhaler (90 µg/puff; 200 puffs/inhaler)	1–4 puffs every 4–6 hours[3]	Preferred formulation in most cases. Clinically similar to metaproterenol but slightly longer duration of action.
	Nebulized solution (0.5%)	0.5 mL plus 2.5 mL normal saline every 4–6 hours[3]	Administer with powered nebulizer or, rarely, by IPPB.
	Unit dose solution (0.083%)	One 3 mL dose every 4–6 hours[3]	Administer with powered nebulizer.
	Powder (Ventolin Rotocaps) (200 µg)	One 200 µg capsule every 4–6 hours	Requires Rotohaler to inhale.
	Tablets (2 mg, 4 mg) Syrup (2 mg/5 mL)	2–4 mg orally every 6–8 hours	An extended-release 4 mg tablet is available for use every 12 hours (Proventil Repetab). Volmax extended-release tablets are available in 4 mg and 8 mg strengths.
Salmeterol (Serevent)	Metered-dose inhaler (21 µg/puff; 120 puffs/inhaler)	2 puffs every 12 hours	Long-acting agent for maintenance therapy of asthma. Should not be used for acute relief of symptoms. The most expensive β_2 agonist.
Metaproterenol (Alupent, Metaprel)	Metered-dose inhaler (650 µg/puff; 200 puffs/inhaler)	1–4 puffs every 3–4 hours (or more frequently)[3]	Preferred formulation in most cases.
	Nebulized solution (5%)	0.3 mL plus 2.5 mL normal saline every 3–4 hours[3]	Administer with powered nebulizer or rarely, by IPPB. Also available as single-dose vial.
	Unit dose solution (0.4% and 0.6%)	One 2.5 mL dose every 4–6 hours[3]	Administer with powered nebulizer.
	Syrup (10 mg/5 mL)	2 tsp orally every 6–8 hours	Tremor, nervousness, palpitations common. Oral formulation therefore not recommended.
	Tablets (10 mg, 20 mg)	20 mg orally every 6–8 hours	
Bitolterol (Tornalate)[2]	Metered-dose inhaler (370 µg/puff; 300 puffs/inhaler)	2–3 puffs every 6–8 hours[3]	
	Inhalation solution (0.2%)	1.25 mL every 6–8 hours[3]	
Pirbuterol (Maxair)[2]	Metered-dose inhaler (200 µg/puff; 300 puffs/inhaler)	2 puffs every 4–6 hours[3]	
	Breath-activated metered-dose inhaler (200 µg/puff; 400 puffs/inhaler)	2 puffs every 4–6 hours[3]	
Terbutaline[2] (Brethaire)	Metered-dose inhaler (200 µg/puff; 300 puffs/inhaler)	2–3 puffs every 4–6 hours[3]	
(Brethine, Bricanyl)	Tablets (2.5 mg, 5 mg)	2.5–5 mg orally 3 times daily	Tremor, nervousness, palpitations common. Oral formulation th refore not recommended.
	Subcutaneous injection (1 mg/mL)	0.25 mg subcutaneously; may be repeated once in 30 minutes	Slow onset of action (30 minutes). Not limited to β_2-adrenergic stimulation.
Isoetharine (Bronkometer, Bronkosol)	Metered-dose inhaler (340 µg/puff; 200 puffs/10 mL inhaler)	1–4 puffs every 3–4 hours[3]	

SO: CURRENT MEDICAL DIAGNOSIS AND TREATMENT
1997 AU: TIERNEY ETAL

Table 9–5. Selected drugs for obstructive airway diseases.[1] (continued)

Drug	Important Formulations	Usual Adult Dosage (Stable Patient)	Comments
Isoetharine (cont'd)	Nebulized solution (1%)	0.5 mL of 1% solution plus 1.5 mL normal saline every 2–4 hours	Adminsiter with powered nebulizer or, rarely, by IPPB.
Isproterenol (Isuprel, others)	Metered-dose inhaler (131 µg/puff; 200 puffs/10 mL)	1–3 puffs every 2–4 hours	
	Nebulized solution (0.5%; 1% also available)	0.5 mL of 0.5% solution plus 1.5 mL normal saline every 2–4 hours	Administer with powered nebulizer or, rarely, by IPPB
Epinephrine (many brands)	Metered-dose inhaler (200 µg/puff)	1 or 2 puffs every 2–4 hours	Available without prescription. β_1 and α stimulation limit usefulness.
	Subcutaneous injection (0.1%; 1:1000)	0.3–0.5 mL subcutaneously; may be repeated once in 30 minutes	Use with caution in older patients or those with tachycardia, hypertension, or arrhythmia. No more effective than inhaled β_2 agonist.
Anticholinergics			
Ipratropium bromide (Atrovent)	Metered-dose inhaler (18 µg/puff; 200 puffs/inhaler)	2–4 puffs every 6 hours	More potent than sympathomimetics in COPD. Minimal side effects.
	Unit dose inhalation solution (0.02%)	One 2.5 mL dose every 6–8 hours	
The phyllines			
Theophylline, oral (many brands)	Sustained-release tablets and bead-filled capsules	200 mg orally every 12 hours initially; thereafter, 200–600 mg orally every 8–12 hours	Maintenance dose is guided by serum theophylline level. Therapeutic level is 10–20 µg/mL. Absorption varies with brand. Formulations are also available for administration every 24 hours.
Aminophylline	Intravenous	Loading dose is 5.6 mg/kg over 30 minutes for a person not using oral theophylline; maintenance dose is 0.7 mg/kg/h by constant infusion pump—lower if patient has liver disease or heart failure or is receiving erythromycin or cimetidine.	Seldom indicated. Calculate dose from lean body mass. Monitor serum theophylline level.
CORTICOSTEROIDS			
Beclomethasone dipropionate (Beclovent, Vanceril)	Metered-dose inhaler (42 µg/puff; 200 puffs/inhaler)	2 puffs 4 times daily, or 4 puffs twice daily	Rinse mouth with water after use to prevent oral candidiasis; use 30 seconds after inhaled sympathomimetic to control cough and airway irritation. Spacer devices also helpful to prevent oral candidiasis.
Triamcinolone acetonide (Azmacort)	Metered-dose inhaler with spacer (100 µg/puff; 240 puffs/inhaler)	2 puffs 4 times daily, or 4 puffs twice daily	Cough and wheezing after inhalation are reported to be less than after inhalation of beclomethasone.
Flunisolide (AeroBid)	Metered-dose inhaler (250 µg/puff; 100 puffs/inhaler)	2–4 puffs twice daily	Dosing frequency of twice daily offers an advantage.
Prednisone (several brands)	Tablets (2.5, 5, 10, 20, and 50 mg)	Acute bronchospasm: 40–60 mg (1 mg/kg) every 24 hours Chronic bronchospasm: 5–40 mg daily or every other day	Discontinue after 14 days if possible.
Methylprednisolone sodium succinate (several brands)	Intravenous injection (vials of 40, 125, 500, 1000, and 2000 mg)	0.5–1 mg/kg every 6 hours	Clinical response may be delayed for several hours.

(continued)

Table 9–5. Selected drugs for obstructive airway diseases.[1] (continued)

Drug	Important Formulations	Usual Adult Dosage (Stable Patient)	Comments
Hydrocortisone sodium succinate (several brands)	Intravenous injection (100, 250, 500, and 1000 mg)	4 mg/kg every 6 hours	Clinical response may be delayed for several hours.
ANTIMEDIATORS			
Cromolyn sodium (Intal)	Metered-dose inhaler (800 µg/puff; 200 puffs/14.2 g canister)	2–4 puffs 4 times daily	Clinical response may require 2–4 weeks of treatment. Useful only for prophylaxis; younger patients with asthma are more likely to benefit. To prevent bronchospasm, cromolyn may be used 15–30 minutes before exercise or exposure to cold air or allergens.
	Nebulized solution (20 mg/2 mL ampule)	20 mg 4 times daily by powered nebulizer	
Nedocromil sodium (Tilade)	Metered-dose inhaler (1.75 mg/puff; 112 puffs/inhaler)	2 puffs 4 times daily	Maintenance therapy for asthma.

[1]Only drugs available in the United States are listed.
[2]Preferential effect is on β_2-adrenergic receptors.
[3]More frequent dosing for acute or severe episodes of bronchoconstriction is acceptable.

All these schedules of therapy are aiming at meeting the **goals of asthma therapy**.

Various authors put various goals. In general all of them are aiming at reduction of the patient sufferings and enable him to live a rather normal life, while using a single drug or a combination of various drugs as needed by the particular patient.

Although individuals may have specific therapeutic goals, **general goals include:**

(1) Optimal control of asthma with the use of the least amount of medications possible and minimal side effects.

(2) Reduction in hospitalization and emergency care visits.

(3) Prevention of nocturnal symptoms

(4) Tolerance to physical activity appropriate for the patients age.

(5) Improvement of pulmonary function.

(6) Minimization of lost time from school, work, or daily activities (Ref.)

In a special issue, Allergy, the Journal of the European Academy of Allergy and Clinical Immunology (EAACI) defined the goals of asthma management as follows:

(Allergy Supplement (1995) 27:50)

1. Enable the patient to enjoy a normal life, comparable to that of a healthy person.
2. Maintain respiratory function as a close as possible to normal levels.
3. Prevent cough and dyspnoea at night ensuring a good sleep.
4. Prevent asthma exacerbation.
5. Minimize side effects from asthma,.
6. Reduce mortality.

Other Criteria to approach long term treatment

The goals of asthma therapy are to minimize chronic symptoms that impairs normal activity (includes exercise), to prevent recurrent exacerbation, to minimize the need for emergency department visits or hospitalizations, and to maintain near-normal pulmonary function. These goals should be met while providing optimal pharmacotherapy with the fewest adverse effects (Ref.)

The drawbacks of this conventional therapy are:

(1) Frequent dosage, several times/day.

(2) Side effects are prominent especially with oral drugs.

(3) This conventional therapy fails to meet the goals mentioned, as evident from the presence of persistent asthma of different grade (mild, moderate, sever) with treatment.

(4) Patient compliance is poor due to the chronicity of the illness and frequent use of medication.

(5) Psychological sufferings.

(6) Frequent absence from school and work.

(7) The economic load on the family.

(8) Disruption of family dynamics.

(9)Asthma must be regarded as a potentially life- threatening disorder even in extremely mild cases. The incidence of mortality is increasing. Keeping in mind the huge member of patients involved world wide, it is clear that the problem need an urgent action.

Humanitarian Efforts are Needed to Help the Asthmatics

The World Health Organization's (WHO) 1997 report indicated an increasing prevalence of asthma in the past two decades in both children and young adults probably resulted from environment pollution. Asthma together with a chronic bronchitis and emphysema kill almost 3 million people globally each year. Asthma can be fatal even in young people. The *reasons are poorly understood.*

Asthma is responsible for at least 2% of health-case costs in affluent population. WHO stresses the necessity for developing strategies aimed at reducing the morbidity and mortality in a cost-effective way.

The National Heart Lung and Blood Institute (U.S.A.) and the WHO have jointly instigated a global initiative on asthma (GINA) to design and deliver an effective asthma management and prevention programmes.

With this intention we are targeting a novel site in the immunolopathologenosis of asthma.

Clinical trials show that this inventive therapy has a long-term effect, therefore it will be useful to throw light on the mode of action of drugs used for long term control of persistent asthma.

Asthma Prophylactics

Mast cell stablizers

are safe drugs

(1)disodium cromoglycate (Intal) spincaps for inhalation nedocromil sodium (Tilade) prevent early and late allergic reactions. They work preventively to inhibit information and antigen increase in airway hyperactivity- maintenance therapy requires 3 or 4 daily doses and it can take as long as 4 weeks for an effect to be appreciated. Therapy should be continuos

(2)ketofiren (zaditen).

Properties/Actions

Is a non-bronchodilator anti-asthmatic drug with marked anti-anaphylactic properties and a specific antihistamine effect.

Laboratory experiments, both in vitro and in vivo, have revealed the following properties of zaditen, which may contribute to its anti-asthmatic activity:

* Inhibition of both the acute bronochoconstrictor response to PAF (Platelet Activating Factor) and of PAF-induced airway hyperrespon-siveness.
* Inhibition of PAF-induced accumulation of eosinophils in the airways.
* Inhibition of the release of such chemical mediators as histamine and leukotrienes.
* Antagonism of acute bronchoconstriction due to leukotrienes.
* Reversal or prevention of experimentally induced isoprenaline tachy-phylaxis.

In addition, zaditen excerpts powerful and sustained H_1 receptor blocking activity which can be clearly distinguished from its anti-anaphylactic properties.

3)Corticosteroids: are extremely potent anti-asthma agents. There are severally topical formulations delivered by metered dose inhalers. Usual maintenance dose is 100-200 microgram twice daily.(conns)

Action of Corticosteroids:

Corticosteroids such as prednisolon interfere at many points in the immune response, i.e. lymphocyte recirculation, inhibit neutrophil adherence to vascular endothelium in

an inflammatory and suppression mononcyte macrophage functions such as microbicidal activity and response to lymphokines. (Davidson chapter immunologic disease).

Four weeks treatment with recommended dose was associated with significant improvement in peak flow, FEV1 & rescue Salbutamol use in asthmatic subjects . but **was not** associated with large reductions in markers of eosinofilic inflamation , broncovascular permiability , or **mucus hypersecretion .**

Effect of low- dose Beclonethosone dipropionate on asthma control and airway inflamation. (Fahy- JV; BOUSHEY- HA
Eur- Respir- J. 1998 June;
11(6): 1240- 7)

References:

1. WHO Report 1997
2. PRESS
3. Internal medicine (3)
4. Clinical medicine (P 786 , P 163)
5. Robbins (P 196)
6. Roit (313 / 314)
7. A B Kay a
   b 1
   (P 14 , P 350)

A Breakthrough in the Treatment of Asthma and Allergy by a Novel Use of an Old Drug Possibly Leading to a New Hypothesis in Their Immunopathogenesis.

Inventor:

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Abstract:

A drug (N), present in the market, indicated for diseases unrelated to type 1 hypersensitivity, was linked with allergy in a novel way, using it in a non-routine indication and dosage. The theory was build up during the last 4 years in 3 stages. Stage I: concieving the idea of the link between (N) and allergy. Stage II: proving its utility and reduction to practice, by a double-blind placebo controlled clinical trial, involving 120 subjects to assess its beneficial therapeutic effect in various acute and chronic allergies, depending on symptom scoring and chronological events. The treated patients showed marvelous symptomatic improvement and possible long-term effect, hence proceeding to stage III in which, a study was designed to objectively evaluate the therapeutic effect of (N). Nine severe chronic asthmatics receiving maximal doses of anti-asthma drugs and corticosteroids were treated with (N) administered orally according to a schedule of 30 capsules (or what is equivalent) in total, over the whole study period, while continuing their previous therapy. Within 3-4 days of starting (N) therapy, there was more than 65% improvement in their global self-assessment scoring, enabling the patient to discontinue anti-asthma drugs and corticosteroids. Pulmonary function tests revealed a clinically significant increase in parameters reflecting obstructive lung disease. Several months of follow up showed that the chronically disabled patients came to lead a rather normal life with minimal drugs used on need only. There was a tremendous decrease in the quantity of sputum and in its easinophil content.

Such result most likely indicates a selective switching-off of the inflammatory process. It is a breakthrough in asthma therapy and might lead to a new approach in the immunopathogenesis of asthma and allergy. The response of both atopic and nonatopic asthmatic patients to this treatment might give an additional evidence of similarity of both types going with Humbert's suggestions.

Introduction:

Type I IgE mediated hypersensitivity of Coombs and Gell, is the basic harmful immunologic mechanism in different types of acute and chronic allergies including asthma[28], an extremely common disorder. Five per cent of the population are currently symptomatic, and 7-10% of children[4,15], with great physical and psychological sufferings, high hospitalization and death rates[5,7,22].

From the immunopathologic point of view, asthma is considered as multifactorial disease. It develops on the basis of genetic predisposition and involves characteristic sequence of changes in immune functions[38]. The histopathology of both atopic and nonatopic asthma is a chronic desquamative eosinophilic bronchitis[5,30,38], IgE dependent release of mast cell mediators is responsible for extrinsic allergic asthma[15]. T-cell derived lymphokines are intimately involved in the regulation of IgE production[1] and are responsible for immediate and late inflammation. A series of cell to cell interactions mediated through various cytokines are producing the pathologic features, of the disease[13,18,23,26,27,31,33]. A balance of functionally distinct macrophages needs to be maintained to regulate T-cell reactivity in the lung, *atopic asthma is promoted by dysregulation of T-cell mediated mechanisms*[41].

Eosinophils are a key inflammatory cells in asthma, it is pathologically considered as a chronic desquamative eosinophilic bronchitis. There is now a very persuasive evidence that eosinophils are important inflammatory cells in bronchial mucosal damage and hyperresponsiveness[1,8,30]. Inflammation leads to airway obstruction, as a result of oedema, broncho-spasm, mucus hypersecretion with changes in the viscoelastic properties[15,25,32].

Humbert, M. provided evidence for *similarities in the immunopathogenesis of both atopic and nonatopic asthma*, which are clinically distinct, both being mediated by IgE associated with eosinophilic inflammation IL-4 and IL-5 are essential mediators in both types[39].

Various results of inflammation or Factors involved in it, can be addressed as a therapeutic goal. The ultimate test for a new drug is therapeutic efficacy in clinical trial[27].

The goals of asthma management are[18].

1. Enable patients to enjoy normal life, comparable to that of healthy persons.
2. maintain respiratory function as close as possible to normal levels.
3. Prevent cough and dyspnoea at night and ensure good sleep.
4. Prevent asthma exacerbations.
5. Minimise side effects from asthma medication.

Preventive and symptomatic pharmacologic therapies in use at the present time are not fulfilling the above mentioned criteria, in addition to being short acting[19]. What is needed is a radical change in the way we think about the therapeutics of asthma[5,7,21,22,24].

Research has continued to evolve both for the perfecting single class of drug and for a better addressed long-term treatment, although physicians have not disposed of new drugs for over 30 years. *Only with regards to chromones expectations have been disappointing. In fact, the absence of an ideal drug determines the use of the drug with the fewest side-effects*[37]. Leucotriene inhibitors, a group of new drugs are useful in mild to moderate asthma[27,41]. Results obtained in stage II and III of this study showed that drug (N) therapy can fulfill most of the goals of asthma management mentioned above. Treating cases of severe chronic asthma using a schedule of 30 capsules total over 12 months, resulted in :

65-100% reduction in symptom scoring,

clinically significant increase in pulmonary function test parameters,

quantitative and qualitative changes in the sputum with more than 70-90% reduction in eosinophil count in the sputum compared to pre-treatment condition.

The development of this hypothesis occurred in 3 stages:

Stage I - Being a consultant clinical immunologist, suffering from chronic severe allergic rhinitis, asthma and laryngeal oedema, I underwent certain self limiting health problem in around October 1993, after which my asthma improved greatly (serial serum samples, nasal smears, pulmonary function tests preserved). This incident led me to think of a possible theoretical linking of drug (N) with allergy.

I was eager to convert it into a practical applicable form. I managed with stupendous efforts, in sympathy with patient sufferings, to keep this research ongoing and to communicate and consults a number of prominent immunologists, locally and abroad finally being enabled to hope to bring the innovation (documenting the value of drug (N) in allergy and asthma) to light.

Stage II - The primary objective of this stage was to determine the usefulness of drug (N) therapy in allergy, as a new therapeutic approach or as an alternative in cases resistant to traditional therapy. 120 adult subjects of age group (20-68) with various acute and chronic type I hypersensitivity were treated with drug (N). In a double blind placebo controlled trial after an informed consent into the study. All the subjects were of matched age, sex, type and severity of allergic condition.

Design of the Study

1. Diseases involved include seasonal allergic rhinitis, allergic conjunctivitis, chronic urticaria, asthma and laryngeal oedema, (Table 1).
2. The duration of treatment, the total dose received and the schedule of therapy were verified to find the best method of treating various allergies.
3. The patients were evaluated daily regarding symptoms severity over the preceding 24 hours[35]. A global overall evaluation of treatment efficacy was made by the doctor at intervals accorded to patients attendance.
4. The response was recorded according to the onset of a noticeable effect and the degree of symptomatic improvement.

Table 1 : Final global improvement rating of patients in stage II.

	No. of patients	Markedly improved	Moderately improved	Slightly improved	Unchanged	Difficult to evaluate	Dropped from the study
Seasonal allergic rhinitis	25	15 (60%)	7 (28%)	3 (12%)			
Allergic conjunctivitis	8		1 (12.5%)	2 (25%)	4 (50%)		1 (12.5%)
Chronic urticaria	6		1 (16.6%)	1 (16.6%)	2 (33.3%)	2 (33.3%)	1 (16.6%)
Asthma	15	9 (60%)	4 (26.6%)	1 (6.6%)	1 (6.6%)		
Laryngeal oedema	6	2 (33.3%)	3 (50%)		1 (16.6%)		
Total	60	26 (43.3%)	16 (26.6%)	7 (11.6%)	8 (13.3%)	2 (3.3%)	2 (3.3 %)

5. All the patients included were having severe symptoms which are sufficiently troublesome to interfere with daily activity or nocturnal sleep.

The final global improvement rating includes[25].

* **Markedly improved:** almost approaching normal condition.
* **Moderately improved:** having mild symptoms.
* **Slightly improved:** having frequently troublesome symptoms but not sufficiently interfere with daily activity or nocturnal sleep.
* **Unchanged:** remain as in the pretreatment condition.
* **Difficult to evaluate:** no conclusion could be reached.

6. Three main symptoms were chosen for each of the conditions studied, they were:
   * **In seasonal allergic rhinitis:** running nose, frequency of sneezing, nasal obstruction[28,35].
   * **Allergic conjunctivitis:** redness of the eye, itching, swelling[20].
   * **Chronic urticaria:** frequency of recurrence, distribution on the body, severity of itching[17].
   * **Asthma:** dyspnoea, wheeze, cough[18].
   * **Laryngeal oedema:** fullness in the throat, hoarseness of the voice, inspiratory difficulty.

The data collected depending mostly on symptomatic improvement and chronological events, resulted in arriving at a clear idea of the usefulness of the invention as a practical approach in the treatment of these patients. The results of treatment were impressing and having immense advantages over the presently available drugs, being orally administered, for children and adults, simple schedule of treatment, has no sedative effects, no tachycardia or tremor, with few side effects mentioned in the manufacturer's leaflet. During drug (N) treatment it was possible to stop all other forms of therapy, including steroids. Long term prophylactic effect was noticeable.

In order to get objective evidence of the therapeutic usefulness of drug (N), we proceed to Stage III.

Stage III - Since August 1995, nine patients with chronic severe asthma[3,18], all of whom were on a maximal dose of bronchodilators and maintenance corticosteroids were chosen on account of poor response to conventional treatment, were treated with drug (N), and followed up, till now, as convenient for the patient and feasible in our difficult health service situation with the blockade. Their demographic data are being shown in Table 2.

Design of study:

* Day (0): is considered as baseline. The patients were receiving maximal dose of anti-asthma drugs and corticosteroids.
* Day (1): is the beginning of drug (N) treatment the patients received the recommended oral dose in addition to previous therapy.
* The total amount of drug (N) used is 30 (thirty) capsules, or what is equivalent during the whole study period. They were asked to refrain from taking their conventional drugs when possible.
* The study parameters are presented as Absolute numbers to be compared with pretreatment assessment.

	Age (years)	Sex	Duration of illness (years)	Family History	Type of Asthma	Date of starting treatment	Other allergies	Hypo sensitization	Skin test	ketotifen Intal (DSCG)	other illness	Blood sample available
Nida	42	F	3	+ve	Extrinsic	oct.1993	rhinitis conjunctivitis Larangeal oedema	No	+ve	ketotifen 6 months	No	3
P1 NJ	58	F	32	Father + step son+	Extrinsic	1/8/95 (20 month)	conjunctivitis ear	for 1 year no response	+ve tree pollen penicillin		Diabetis	14
P2 ZK	58	F	32	-ve	Intrinsic disappear on north.	18/10/95 (18 month)	No	some improvement	+ dust mite fish beans	Intal 2 months no response	Diabetis hypertension ischaemic H D	2
P3 FZ	48	F	13	18 year till now 3 sons up to 10 years Father	Extrinsic	12/9/95 (19 month)	chemically Induced	NO	house dust mites	Intal effect similar to puff	Diabetis thyrotoxicosj	3
P4 AS	62	M	25	- ve		4/1/97	- ve	NO	NO	Zaditen	NO	7
P5 HS	45	M	5	+ ve		23/12/96		NO		improve	NO	9
P6 AG	72	M	32	3 daughters	Intrinsic	20/8/95	NO	NO	- ve	NO	NO	1
P7 ZU	48	M	1	- ve	Intrinsic	17/8/95	NO	NO	NO	no response	NO	2
P8 MD	36	F	4	- ve		10/8/96	rhinitis conjunctivitis	NO	NO	Ketotifen useful	NO	
P9 IB	60	M	3	+ ve		14/4/97	NO	NO	NO	NO	NO	5

Table 2 Demographic data of patients involved in stage III

* All patients were advised to stop their corticosteroids and anti-asthma drugs where possible.

Assessment Criteria

1. Symptom scoring:
   a- Symptom triad of dyspnoea, cough and sputum scoring, the maximum score for each is 3. Scores for dyspnea were assessed as: no dyspnoea (score 0), mild on doing physical activity (score 1), moderate at rest (score 2), severe constant annoying dyspnoea (score 3). Scores for cough were assessed as: no cough (score 0), mild cough sometimes (score 1) moderate frequent annoying cough (score 2), severe constant distressing cough (score 3). Scores for sputum were assessed as, no sputum (score 0), small amount expectorated with ease (score 1), tenacious moderate amount (score 2), plenty causing severe mucus-related symptoms (score 3).
   b- Composite symptom scoring: this is an indication of therapeutic effectiveness in improving the patients global assessment[25,30]. The sum of the score is 39, three is the maximum for each symptom, which includes cough frequency, cough severity, ease in bringing up sputum, audible wheeze, tachycardid, chest discomfort, nocturnal dyspnoea disturbing sleep, ability to go upstairs, ability to talk and laugh, stress incontinence caused by the cough, missing days at work, psychological well being. hospitalization rate and need for I.V. drugs.
2. Five patients were able to do serial complete pulmonary function tests (PFT). Using Autospiror (Discom 14, Chest Corporation, Tokyo, Japan). P.F.T. before starting drug (N) therapy was considered as a base line. Daily when possible during the first week and according to patients attendance later on, aiming at finding whether drug (N) is having a bronchodilating effect by evaluating the changes in forced expiratory volume 1st second (FEV1), peak expiratory flow rate (PEFR) at 25%, 50% and 75% of vital capacity (FEF 25%, FEF 50%, FEF75%).
3. Serial microscopical sputum examination for the percentage of eosinophils in relation to other inflammatory cells. Samples were smeared on slides, fixed with methanol, stained by haematoxylin - eosin. A total of 300 inflammatory cells were counted in each slide and the percentage of eosinophil calculated (slides are preserved).
4. Serial serum samples kept at -20 °C, awaiting reagents availability to carry out certain relevant Laboratory tests as total serum IgE (PRIST, Pharmacia, Upsala, Sweden), Ostecalcin (Pharmacia) to follows the systemic effect of corticosteroid treatment, Eosinophil Cationic Protein (Pharmacia) to measure eosinophilic inflammation, follow the efficacy fo drug (N) treatment and monitoring patients compliance, and others.

Results:

1. Symptom scores:

a- Symptom triad: the three major symptoms of dyspnoea, cough and sputum were improved by 67-100%. The improvement started by day 3, to reach maximal within 7-10 days, as shown in figure (1-3 and 10-12). The improvement lasted over the whole study period, with few mild attacks of short duration.

b- Composite symptom scores: the score were improved by 67-100%. The improvement started by day 3, to reach maximal within 7-10 days. As shown in Figures (4 and 13). The improvement lasted over the whole study period with only few mild attacks. They are now free from multiple drug therapy, breath better, leading more comfortable lives, and staying out of the hospital.

2. Changes in pulmonary function test (PFT) parameters:

The bronchodilating effect (more correctly, alteration in airway flow and bronchial patency, resulting from drug (N) therapy, was evaluated as absolute changes in FEV1, PEFR, FEF 25%, FEF 50%, FEF 75% over base line during a period (20-25) days Figs.(5-9) and (14-18) and according to patients attendance later on.

P3: FEV1 increased by 39.7%, range 4.7% - 53.76%) (Fig. 5).
increment in FEF 25% was 49.73%, range (30.0% - 63.16%) (Fig. 9).
increment in FEF 50% was 56.83%, range (0.0% - 66.04%) (Fig. 8).
FEF 75% increased by 38.04%, range (29.41% - 46.96%) (Fig. 7).

P2: increment in FEV1 was 18.09%, range (8.69% - 22.22%) (Fig. 5).
FEF 25% increased by 22.84%, range (-15.38% - 40.0%) (Fig. 9).
The increment in FEF 50% was 34.17%, range (12.82% - 49.25%) (Fig. 8).
The increment in FEF 75% was 63.39%, range (56.25% - 68.18%) (Fig. 7).

P1: increment in FEV1 was 4.42% (range 2.32% - 6.67%) (Fig. 5).
increment in FEF 25% (alveolar) (Fig 9) was 43.49%, range (3.33% - 59.15%).
FEF 50% (small airways) (Fig 8) increased by 26.65%, range (-5.75% - 44.24%).
FEF 75% (large airways) (Fig 7) increased by 43.81%, range (6.45% - 55.16%).
Clinically significant changes in PEFR were also noticed.
Patients 8 and 9 are also presented.
The changes are maintained during the whole study period.

3- Sputum changes:

Macroscopically there was a tremendous decrease in the amount of sputum, it became thin easily expectorated, changes started by day 2 and within 5-7 days the patients were free of mucus related symptoms.

The percentage of eosinophils to other inflammatory cells decrease from 80% to less than 10% within the 1st two weeks.

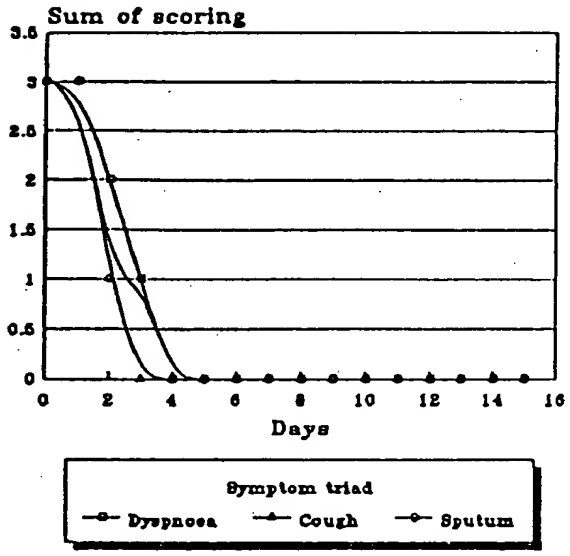


Fig.(1):Results of symptom triad scoring for patient (1) during drug (N) therapy

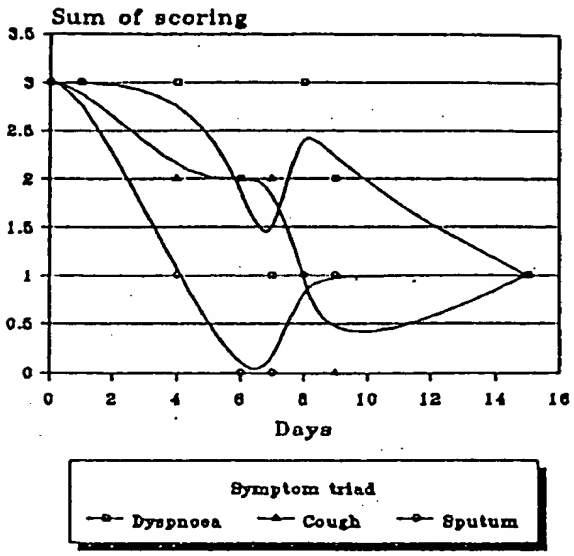


Fig.(2):Results of symptom triad scoring for patient (2) during drug (N) therapy

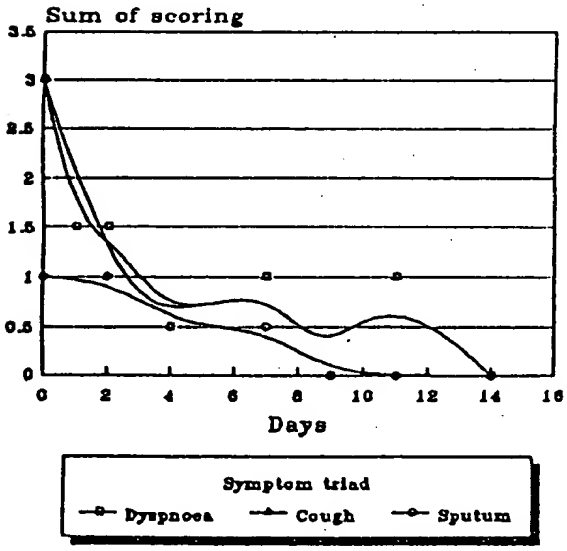


Fig.(3):Results of symptom triad scoring for patient (3) during drug (N) therapy

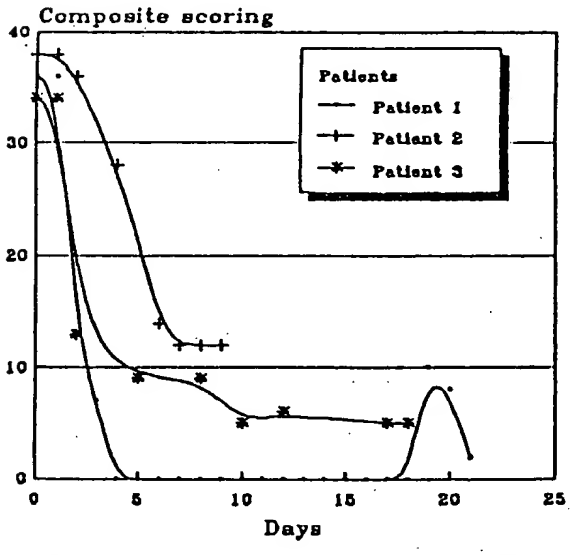


Fig.(4):Results of composite symptom scoring of the three patients

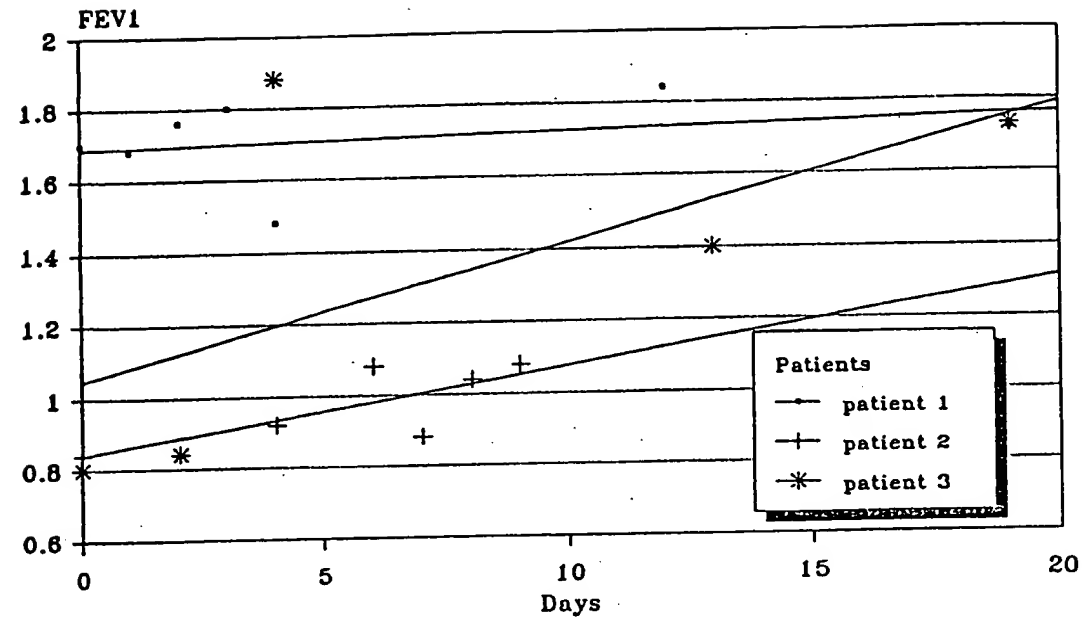


Fig.(5):Changes in FEV1
produced by drug (N) therapy
in the three patients

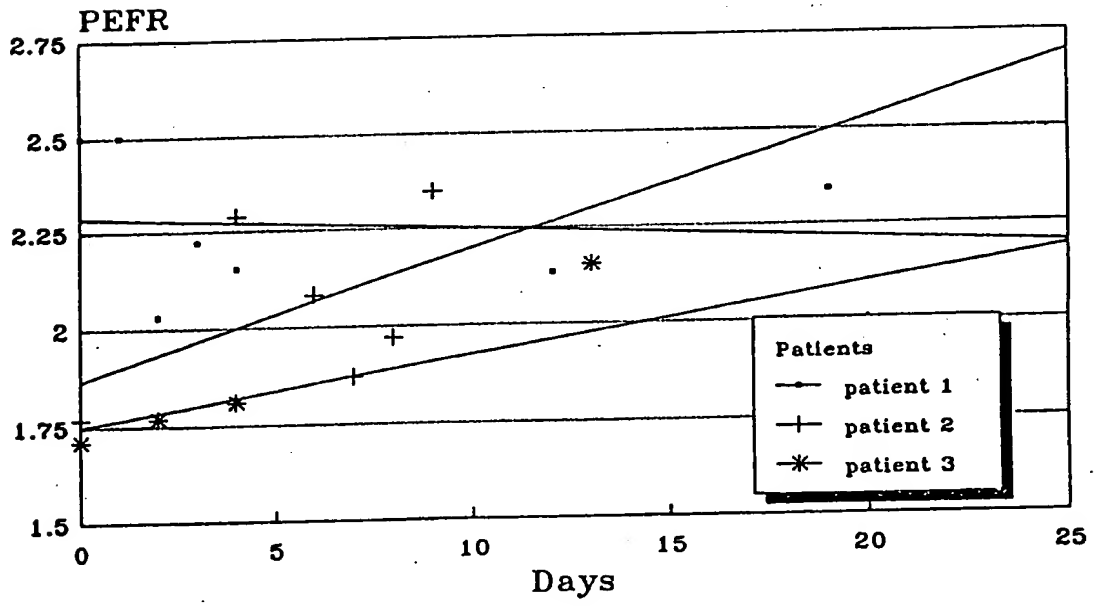


Fig.(6):Changes in PEFR
produced by drug (N) therapy
in the three patients

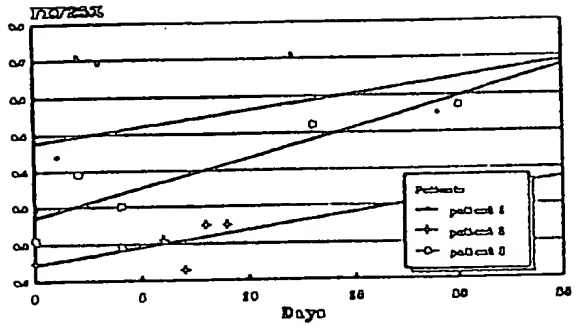


Fig.(9):Changes in FEV25% produced by drug (N) therapy in the three patients

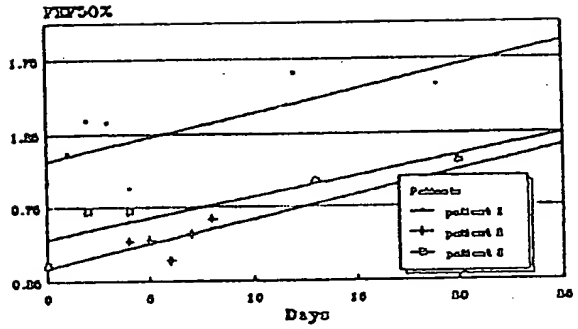


Fig.(8):Changes in FEF50% produced by drug (N) therapy in the three patients

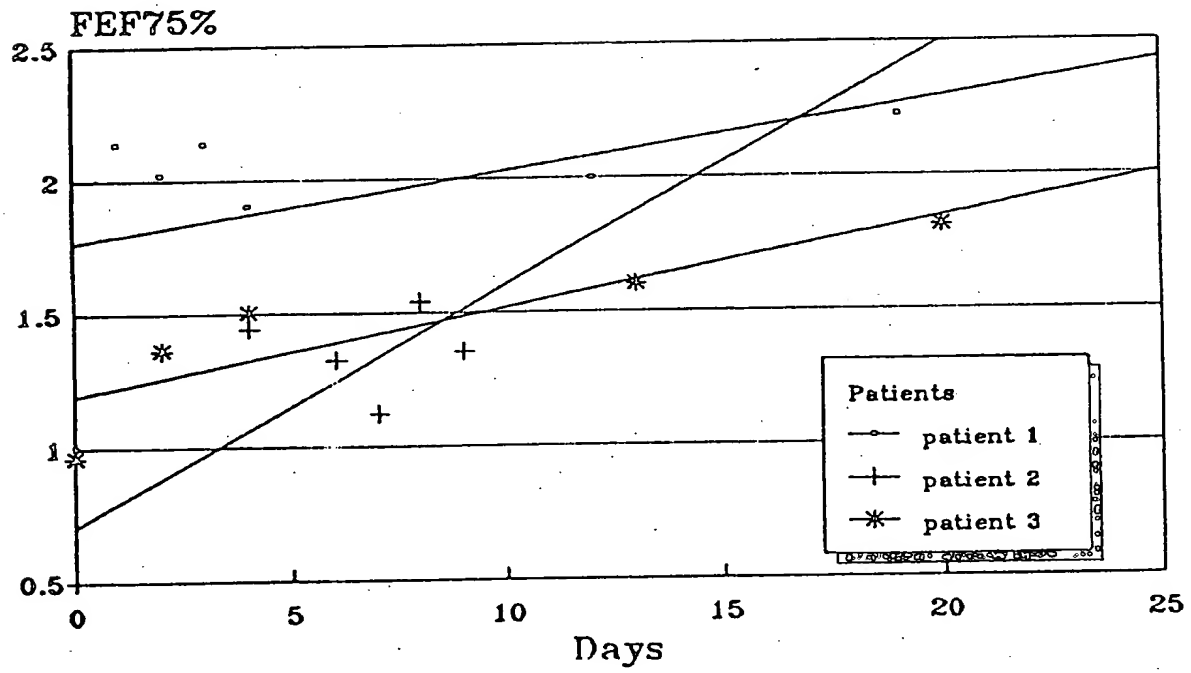
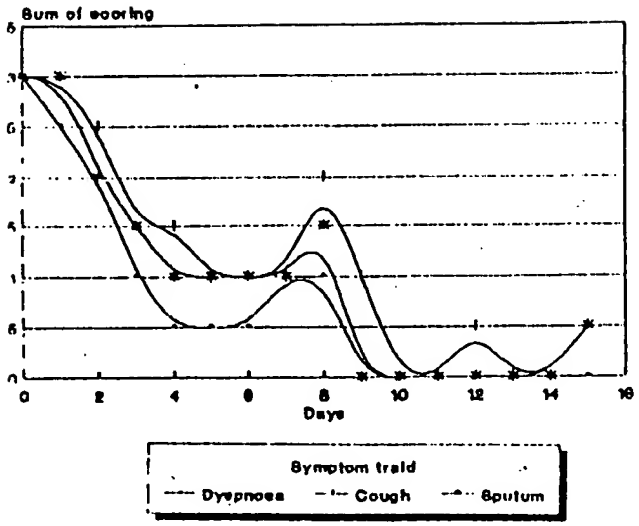
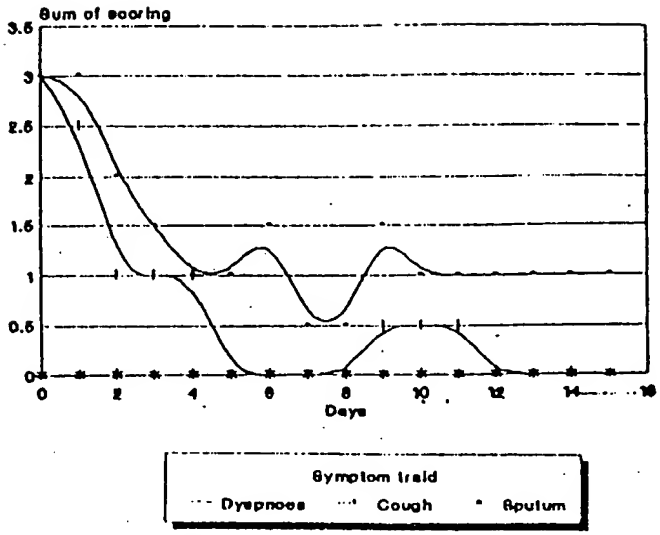


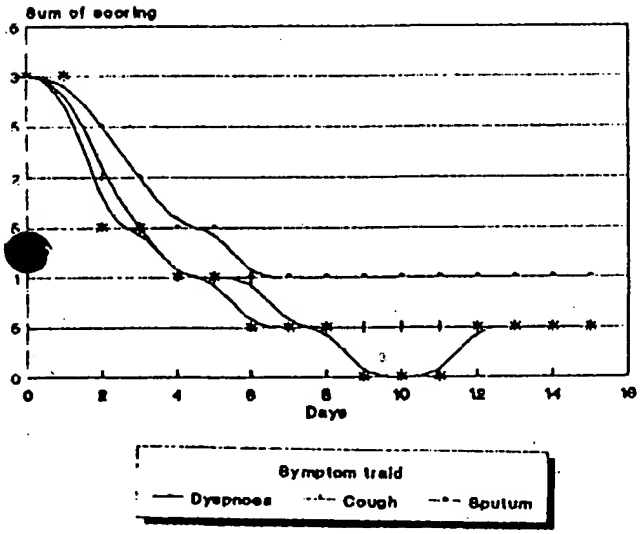
Fig.(7):Changes in FEF75% produced by drug (N) therapy in the three patients



Fig(10) Results of symptom traid scoring for patient (4) during drug (N) therapy



Fig(11) Results of symptom traid scoring for patient (5) during drug (N) therapy



Fig(12) Results of symptom traid scoring for patient (9) during drug (N) therapy

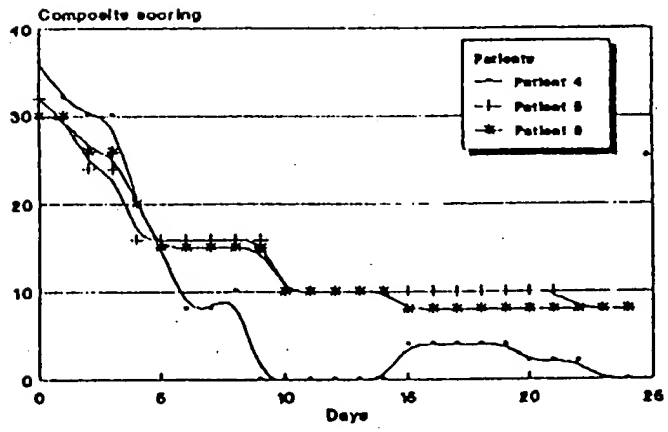
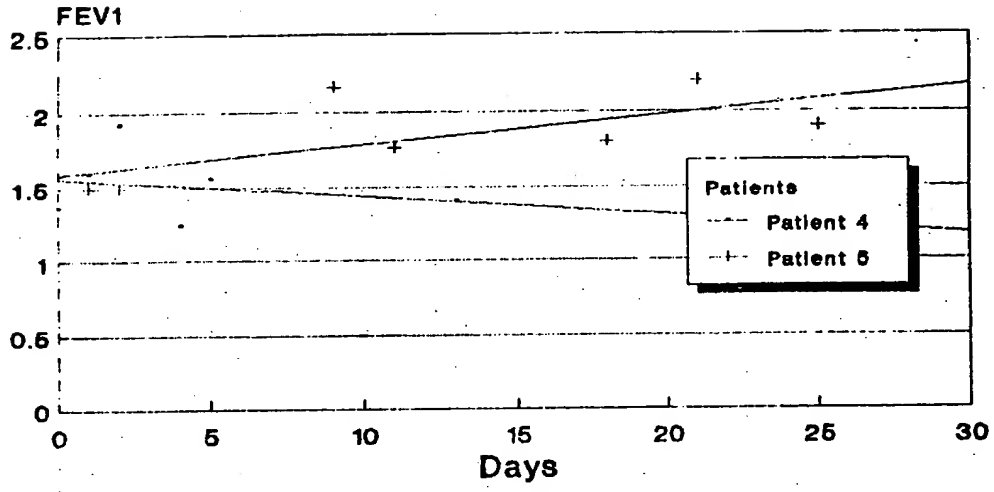
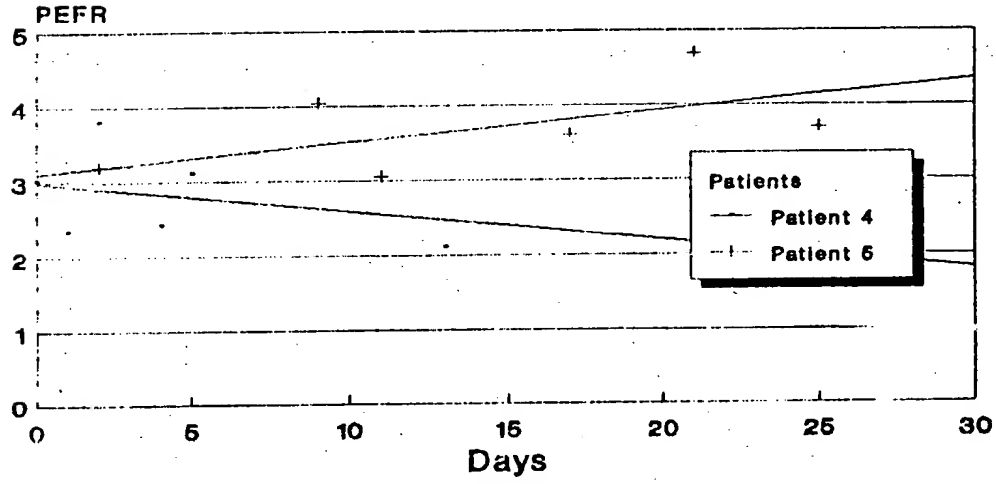


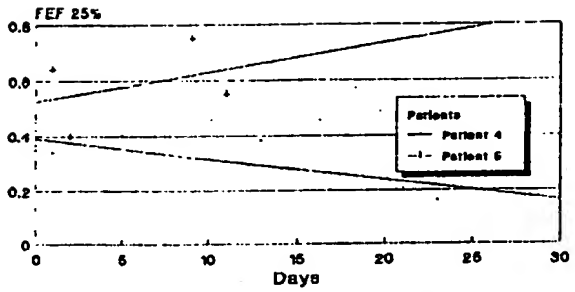
Fig (13)Results of composite Symptom scoring of P4 , P5 & P9



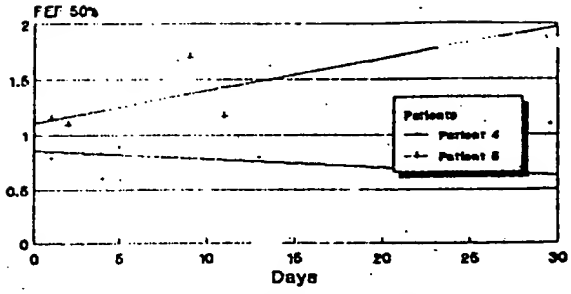
Fig(14)Changes in FEV1
produced by drug (N) therapy
in P4 & P5



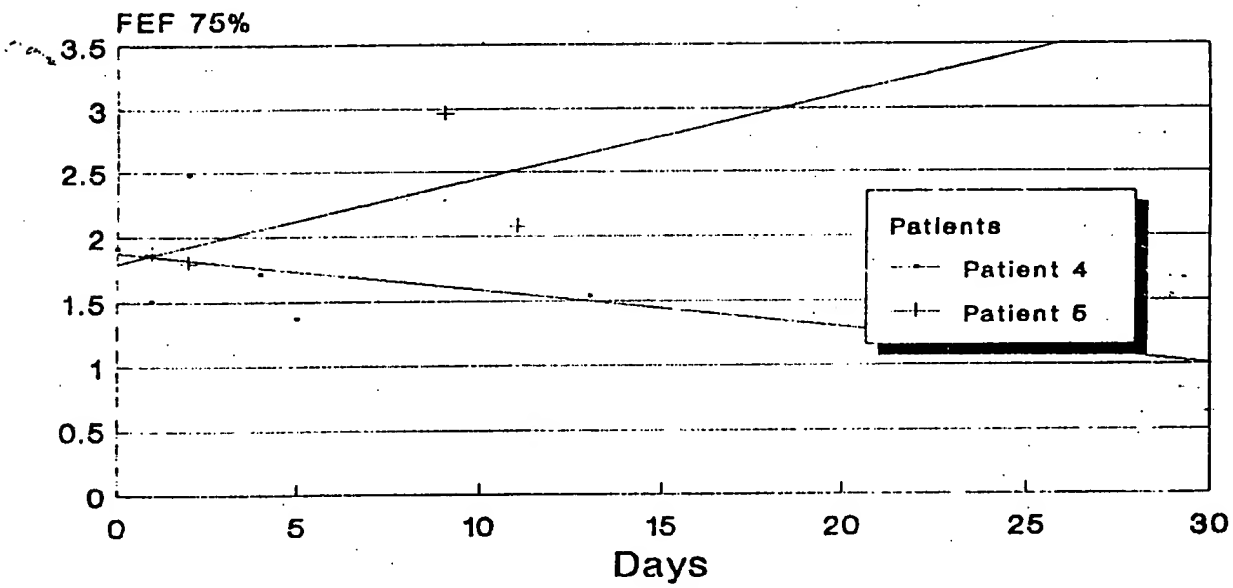
Fig(15)Changes in PEFR
produced by drug (N) therapy
in P4 & P5



Fig(18)Changes in FEF 25% produced by drug (N) therapy in P4 & P5



Fig(17)Changes in FEF 50% produced by drug (N) therapy in P4 & P5



Fig(16)Changes in FEF 75% produced by drug (N) therapy in P4 & P5

4-Changes in the serum during the study period need to be unmasked later, after carrying the appropriate laboratory tests.

Long Term Follow-Up

The patients involved in stage III had been followed-up for varying periods starting Aug. 1995. The total dose of drug (N) received is thirty (30) capsules or what is equivalent, no additional dose was given.

The assessment criteria include: 1. Rate of hospitalization, 2. The need for concomitant traditional therapy, 3. The frequency of attacks of shortness of breath, cough, wheeze and sputum, 4. Daily activity, 5. Disturbance of sleep.

The follow up showed that the hospitalization rate was reduced from several times *per month* to 1-3 times *per year*, they needed concomitant therapy when they got a cold only, the asthmatic attacks we very few and lasted for a shorter time and were much milder ,manifested mainly as shortness of breath, mild cough, *scanty or no sputum.* Eight of ten were able to live a rather normal life, night and early morning dyspnoea disappeared, no mortality was recorded among them.

The sputum is absent all the time and only very scanty during acute exacerbation. Microscopical examination showed that the reduction in eosinophils number / compared to other inflammatory cells was 5-10% and was maintained except during exacerbation where it rises to 30-40% for a short period.

Serum samples taken at varying intervals were kept in (-20 °C).

It had been noticed that drug (N) treatment got a preventive and long term effect, particularly in seasonal allergic rhinitis, laryngeal oedema and asthma and might lead to a new hypothesis in the immunopathogenesis of type I hypersensitivity.

Discussion:

It is well known that asthma is a common disease. A wide spectrum of anti-asthma drugs are available, some are treating the symptoms, and others aiming at the immunopathologic factors involved. Any of these drugs, singly or in combination, are not fulfilling the goals of asthma management[18] in many patients. A continuous search for a new better drug is going on[4,5,12,22,24,26,27]. *The ultimate test for the therapeutic effect of a new drug is clinical trial*[27].

In our present hypothesis, after the theoretical linking of drug (N) with allergy, a clinical trial was carried out, to evaluate the therapeutic effect and reducing it to practice. During stage II we depend mainly on subjective improvement and chronological events. The results were greatly impressing. In stage III, 9 patients were treated by drug (N) according to a schedule of 30 capsules in total the assessment criteria were subjective improvement in addition to studying the effect on objective parameters. Assessment involves.

1. Symptom scoring

a- Symptom triad of the three cardinal symptoms, dyspnoea, cough and sputum.

b- A more comprehensive global self assessment scoring involving 13 symptoms (composite symptom score)[20,25].

The results of both assessments was a 65-100% reduction in the score started by day 3 to reach maximum in day 10 and was maintained during the study period of 13-10 months. A search in database (MEDLINE silver platter 3.11) up to May. 1997 showed no similar therapy, therefore, such results are novel.

2. The second assessment criteria was a complete pulmonary function test, to assess airway patency and detect "how much" of a bronchodilating effect does drug (N) treatment produces.

FEV1 is a valuable screening procedure for obstructive lung diseases. It is also useful in assessing the efficacy of bronchodilator therapy and in following the progress of the patient with asthma, 15% increase in FEV1 over baseline is considered clinically significant[2,15]. In our patients the percent increment in FEV1 over the base line, during the 20 days period (Fig. 5) in more than 15%, so it has got a significant bronchodilatating effect. Changes actually started after 4-6 days therefore, it seems that the increment in FEV1, the alteration in airway flow and bronchial patency is the result of an effect on the basic immunologic inflammatory process.

In P3 FEV1 increment was evident by day 4 (57.47% increase from base line and maintained later, but the day 2 reading (4.76%) lowered the mean.

Regarding P2 mean FEV1 was lowered by delay onset of action and by a low reading by day 7 (4.55% increase from base line) due to the natural course of the disease. Otherwise the % increment was around 20% patient was off- corticosteroids and continued to need bronchodilator tablets and puffs on and off.

In P1. FEV1 base line value was very high (more than 75% of predicted) and was maintained during the study period. There was a marked lowering by day 4 (-13.51), which Negatively effect the mean FEV1 increment, otherwise, it will be about 22.28%.

The increment in forced expiratory flow at 25% of vital capacity (FEF 25%) (alveolar), FEF 50% (small airways), FEF 75% (large airways) was tremendous. An increase of 20-25% from base line is considered clinically significant. The results in the patients were about or, 2-3 folds, this value. Clinically significant PEFR changes were also observed. Patients 8 and 9 showed similar changes.

Together with the other highly positive changes in the various assessment criteria, is reflecting a possibility that their is a gradual switching-off of the inflammatory process. *Such increment in the various parameters is totally unexpected.*

3. Sputum changes:

Hypersecretion of mucus causing mucus related symptoms (25) and changes in its viscoelastic properties, are characteristic of asthma. During drug (N) treatment there were a marked decrease in the quantity and a change in viscoelastic properties, clinically evident by 3 days. Most of mucus related symptoms disappeared by day 10.

Eosinophils are a key inflammatory cells in asthma, it is pathologically considered as a chronic desquamative eosinophilic bronchitis. The number of eosinophils in the sputum is correlated to the severity of the disease. Ellinor A. et. al.[9] has shown that the total cell number, or differential cell counts in either the bronchial wash or the bronchoalveolar lavage fluid, before and after treatment with budesonide or terbutaline, showed no significant chanes. Patricia D. et. al.[25] had shown that 28 days treatment with cromolyn 40 mg (two spincaps) four times daily, resulted in a significant decrease in the percentage of eosinophils in bronchial mucus in the responder group. Zaditen (Ketotifen) is inhibiting the accumulation of activated eosinophils[30].

In this study sputum examination shows that there was more than 70-90% reduction in the number of eosinophils related to other inflammatory cells. Upon correlating the reduction in the number of eosinophils per unit volume of sputum, with the daily sputum output, the reduction in eosinophil number was remarkable. Such results bear great similarity to drugs that are treating the underlying cause of asthma, and will possibly lead to a new approach in the immunopathogenesis of asthma.

Acknowledgments:

I would like to thank *Dr. Akram Abbood* for statistical help, Dr. Muna Taki for sputum examination, Mrs. Nahida Al-Janabi for PFT, and Mrs. Najat Mihsin, Faiza Ali, Zakia Khalid for cooperation.

N. Nassir

Sincerely Yours

Online Articles

Nigella Sativa, Commonly Known As "Love in the Mist" A Beautiful Middle Eastern Herb With Many Uses

Dr. Michael Tierra L.AC., O.M.D.

Nigella
(NIGELLA SATIVA L.) Black Cumin, Fitch (Biblical), Love in the Mist, Fitches
"...For the fitches are not thrashed with a threshing instrument. ..but the fitches are beaten out with a staff..." Isaiah 28

- Parts Used: seeds
- Energy and Flavors: Hot energy, spicy flavor
- Systems Affected: Lungs, Stomach, spleen
- Biochemical Constituents: Alanine, arginine, ascorbic-acid, asparagine, campesterol, carvone, cymene, cystine, dehydroascorbic-acid, eicosadienoic-acid, glucose, glutamic-acid, glycine, iron, isoleucine, leucine, d-limonene, linoleic-acid, linolenic-acid, lipase, lysine, methionine, myristic-acid, nigellin, nigellone, oleic-acid, palmitic-acid, phenylalanine, phytosterols, potassium, beta-sitosterol, alpha-spinasterol, stearic-acid, stigmasterol, tannin, threonine, thymohydroquinone, thymoquinone, tryptophan, tyrosine
- Properties: Stimulant, aromatic, carminative, digestive, diuretic, emmenagogue, excitant, galactatagogue, purgative, resolvent, stimulant, stomachic, sudorific, tonic, and vermifuge

Uses: For me the common name "love in the mist" aptly describes the poetry of this exquisite plant. In the garden, one easily imagines etheric spirits flitting about amongst its evanescent bluish-white blossoms. Even the seedpods, which are so often used in dried flower arrangements, suggest an otherworldly sense of exotic enchantment. Is it possible that such a delicately beautiful herb, with such potent medicinal properties would be so hardy as to easily reseed itself in our gardens year after year?

With an exalted position of use throughout the Middle East and to a somewhat lesser extent in India and other Eastern lands, the information about Nigella I owe to herbalist, plant-scientist extraordinaire, Jim Duke as presented in his book Medicinal Plants of the Bible. In it he describes Black Cumin as a Muslim Miracle Herb which, according to an Arab Proverb it is said that, 'in the black seed is the medicine for every disease except death.'

I have spoken with a Turkish colleague who reports that it the seeds are widely cultivated and traded in ton lots within his country throughout the Middle East, Northern Africa and India. The seeds are used both as a condiment in bread and cakes and various confections and like pepper or combined with pepper such as cayenne in sauces. The Ethiopians add along with other spices to flavor local alcoholic beverages. Still another use is to sprinkle them with woolen garments as a moth repellant.

The major uses I have employed it for are upper respiratory conditions, allergies, coughs, colds, bronchitis, fevers, flu, asthma and emphysema for which it is effective. Simply collect the abundance of seeds from the pods and grind them to a paste and mix with melted honey to a 'hahlava' (a Middle Eastern confection usually made with toasted sesame seeds and honey). Jim Duke confirms its folk use for these and a wide variety of other diseases and conditions including bilious ailments, calluses, cancer, colic, corns, eruptions, headache, jaundice, myrmecia, orchitis, puerperal fever, sclerosis, skin, snakebite, stomachache, swellings, tumors of the abdomen and eyes, and warts. In Algeria, the roasted seeds are combined with butter for cough and honey and taken for colic.

2A

For upper respiratory conditions, at least a few of its constituents have shown an antihistamine-like action, which explains is positive effects for upper respiratory diseases including asthma, bronchitis, and cough. The oils of the seed increase milk flow which explains its folk use as a galactagogue. In large quantities, however, the seeds have also been used to abortion.

It is unusual for a hot spicy herb to have a positive effect on liver diseases as it is used by the Lebanese. Of course, one of its most obvious uses is for diarrhea and dysentery, combined with astringents. Externally the seeds can be ground to a powder, mixed with a little flour as a binder and applied directly to abscesses, on the forehead for headache, nasal ulcers, orchitis, and rheumatism. The seeds also are a rich source of sterols, especially beta-sitosterol, which is known to have anticarcinogenic activity. This substantiates its folk use for indurations and/or tumors of the abdomen, eyes and liver.

In India, Nigella seeds are combined with various purgatives to allay gripping and colic and also help kill and expel parasites. Middle Eastern Unani medicine affirms its abortifacient properties and also use it as a diuretic to relieve ascites, for coughs, eye-sores, hydrophobia, jaundice, paralysis, piles and tertian fever.

Contraindications: Do not take during pregnancy.

2B

A SEARCH FOR AN INDUSTRIAL PARTNER IN DEVELOPING, PATENTING AND MARKETING A NEW ANTI-ASTHMA, ANTI-ALLERGIC DRUG.

Inventor: Dr. NIDA NASSIEF, MD, M.Sc (U.K.)
Consultant Clinical Immunologist and Pediatrician
Central Public Health Lab. Baghdad-IRAQ

Associate Workers: -

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DR. Z.A. FADHEL, MD, DVD (U.K.), Consultant Dermatologist. Yarmook Teaching Hosp.

DR. H.A.G. NASSIEF, MD, D.O., FRCS (U.K.), Asst. Prof. in ophthalmology .Mustansiriya University.

Back during October 1993, certain health incident happened to the inventor, after which, her asthma and laryngeal oedema was tremendously improved. Based on this event, a theoretical speculation of the immunological events that resulted in the improvement was forwarded. Conceiving the idea of the whole research was considered as STAGE I.

Since then, stupendous efforts motivated by patients suffering are continuous to complete the work.

STAGE II: An animal experimentation was designed to make-true one aspect of the theoretical speculation. Four white albino rats were challenged locally intranasaly with certain substance, that is considered as a backbone in the skeleton of the theory .The results of histopathological examination of the nasal cavity of the rat were encouraging, it showed evidence of an *in-vivo* novel eosinophil chemotactic factor. It was concluded that these key inflammatory cells in asthma are related in-a-way to the backbone above mentioned. Fig.1 and fig.2 demonstrates eosinophilic infiltration of rat nasal cavity and submucosa.

STAGE III: A drug (N), present in the market, indicated for diseases unrelated to type 1 hypersensitivity, was linked (depending on the above speculation) with allergy in a novel way, using it in a non-routine indication and dosage.

In order to prove its utility and reduction to practice, a double-blind placebo controlled clinical trial, involving 120 subjects (60 patients treated with (N) and 60 patients matched for age, sex, and severity of the allergic condition were treated with placebo) to assess (N) beneficial

therapeutic effect in various acute and chronic allergies, depending on symptom scoring and chronological events.

1.Diseases involved includes seasonal allergic rhinitis, allergic conjunctivitis, chronic urticaria, and asthma and laryngeal oedema.

2.The duration of treatment, the total dose received and the schedule of therapy were verified to find the best method of treating various allergies.

3.The patients were self-evaluated daily regarding symptoms severity over the preceding 24 hours. A global overall evaluation of treatment efficacy was made by the doctor according to daily notes at intervals, depending on patients attendance.

4.The response was recorded according to the onset of a noticeable effect and the degree of symptomatic improvement.

80% of the treated patients showed totally unexpected marvelous symptomatic improvement particularly seasonal allergic rhinitis, asthma and laryngeal edema.and a long-term effect were noticed, hence proceeding to: -

STAGE IV: in which, a study was designed to *objectively* evaluate the therapeutic effect of (N). Since Aug. 1995, nine severe chronic asthmatics receiving maximal doses of anti-asthma drugs and corticosteroids, chosen on acount of poor response to conventional treatment were treated with (N), administered orally according to a schedule of 30 capsules (or what is equivalent) in total, over the whole study period, while continuing their previous therapy. Assessment was carried out by using:

- Symptom scoring

  One-Symptom triad of the three cardinal symptoms, dyspnoea, cough and sputum.

  b-A more comprehensive global self-assessment scoring involving 13 symptoms (composite symptom score).

The results of both assessments was a 65-100% reduction in the score started by day 3 to reach maximum in day 10 that was maintained during the study period of several months to few years. The patients were enabled to lead a rather normal lives, with minimal convential anti asthma drugs and odiscontinue corticosteroids, Fig.3, Fig.4 shows the pattern of changes in symptom scoring.

- The second assessment criteria was a complete pulmonary function test, to assess airway patency and detect "how much" of a bronchodilating effect does drug (N) treatment produces. FEV1, PEFR, FEF 25%, 50% and 75% are valuable screening parameters for obstructive lung diseases. They are useful in assessing the efficacy of bronchodilator therapy and in following the progress of the disease.

Drug (N) therapy results in a clinically significant increase in parameters reflecting obstructive lung disease. The amplitude of the changes are greater than what is produced by a bronchodilator drug.

- Sputum changes: During drug (N) treatment there were a marked decrease in sputum quantity in addition to a change in viscoelastic properties, clinically evident by 3 days. Most of mucus related symptoms disappeared by day 10.

Serial microscopical sputum examination shows that there was about 70-90% reduction in the number of eosinophils related to other inflammatory cells reached by day (14). Upon correlating the reduction in the number of eosinophils per unit volume of sputum, with the daily sputum output, the reduction in eosinophil number was remarkable. Such results bear great similarity to drugs that are treating the underlying cause of asthma. Fig.5 and fig.6 demonstrate the reduction in sputum eosinophils.

- Serum changes: Serial serum samples from patients were taken and stored at (-20) awaiting reagent's availability to unmask the changes in relevant parameters.

Serial samples from stage I are also stored.

A NEW HYPOTHESIS in the immunopathogenesis of type I hypersensitivity were built up.

The corner stone is the tremendous reduction in sputum eosinophils that was maintained for very long period, temporarily abolished during acute exacerbation. It occurs even in none-responder group.

Other stones of the new hypothesis are:

- The delayed onset of action.
- The pattern of the changes in symptom scoring.
- The amplitude of changes in the relevant parameters of pulmonary function test.
- Long term effect (years).
- The response of both extrinsic and intrinsic asthma

All of them indicate a novel unpreceded-till now-*selective switching off* of the eosinophilic airway inflammation. Hopefully this will unmask important scientific facts in the immunopathogenesis of allergy and asthma that will at the end pave the road for a new antiasthma era.

Information search regarding patent laws showed that there is an Australian law that applies to patenting new medical treatments. (Louphlan P.L; Med. J. Aust. (1995) 3: 162). Therefore, during early 1997, the Australian Industrial Property Organization (AIPO) lit a candle in the research way. Their opinion stated that "it appeared from the details you provided, that you have sufficient experimental results for a patent application". Their advice was to contact the World Intellectual Property Organization (WIPO). Copies of the research results were sent to WIPO and the Department of Health and Human Services, Food and Drug Administration (FDA) for evaluation. Thankfully, FDA forwarded the copy to the center for drug Evaluation and Research for review as a New Drug Application (NDA) *without*

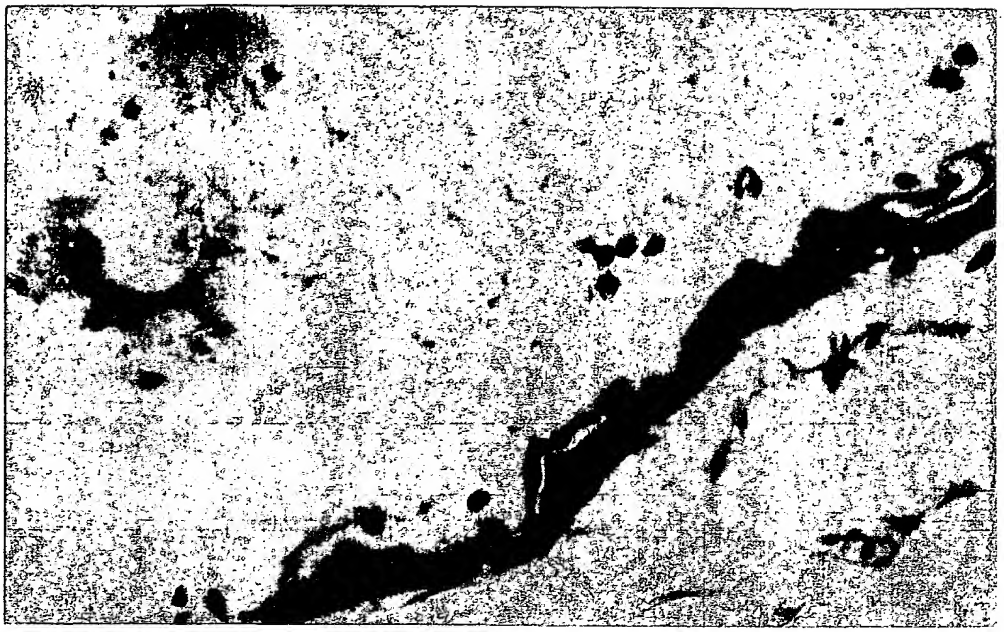

Fig. 1, above : A microscopical view of a section of rat nasal cavity and mucosa stained with haematoxyline –Eosin (H & E) magnification X 400, showing evidence of eosinophil chemotaxis (arrowed).

Fig. 2, below : Eosinophil infiltration of the submucosa.

Fig. 5, above : Microscopical view of patients sputum H & E stained X 400, *befor* drug (N) therapy , The field is full of eosinophils , *Owl eye like appearance* .

Fig. 6, below : Same patients sputum , *after* treatment,There are very scanty eosinophils and reduction in inflammatory cells. Carbon–Laden macrophages and fibroblasts are seen.

unmasking Drug (N) identity. A patent attorney was consulted, and after a careful search, he found that the invention is novel.

After all these stupendous self supported efforts of years we are still far away from providing this miracle in-between the hands of the suffering patients, while being able to preserve our rights.

STAGE V: since late 1997 and during 1998 efforts were continuous to develop an **ALTERNATIVE SUBSTANCE (A)**, which can be patented, has it's own identity and trade name, in order to replace drug (N). Before hand the immunological action of substance (A) was studied in vitro. Its beneficial effect to allergy was accidentally noticed. Therefore, depending on these informations, a clinical trial was started during July 1998.The available clinical data showed similarity of anti-asthma effect of the alternative substance (A) with the previously used drug(N).

Substance (A) has got the following characters:

1. It is orally administered in a simple schedule similar to drug (N).
2. There is evidence that (A) or its metabolites are excreted in the urine.
3. Some aspects of its mode of action has been studied in vitro.
4. Is considered to be safe as it had been used by human beings since the ages.
5. It is not registered under any trade name, most likely it will be a patentable invention.
6. It seems that *the secrete lies in the schedule of therapy* as well as in the mode of action of substance (A) and drug (N).

At present we are in urgent need for an industrial partner to take over the completion of the invention and sponsoring patenting a marketing. With my best regards and hopes for dual cooperation.

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I would like to thank Dr. Muna Taki for sputum examination, Mrs. Nahida Al-Janabi for PFT, Dr. Nabil Abu-Zaid for lab. Assessment of substance (A),Dr. *Akram Abbood* for statistical help, Dr. Najah Mohd Ali, and other helping colleges.

Mrs. Najat Mihsin, Faiza Ali, Zakia Khalid, and Mr.Abdul-satar for cooperation.

Sincerely Yours

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Although a variety of changes can be measured, lymphocyte responsiveness is most commonly assessed by measuring DNA synthesis through the incorporation of radiolabeled thymidine (3).

And it has been found that the amount of DNA synthesis correlates with the percent of cells becoming blastlike. DNA synthesis is measured by the incorporation of 3^H-thymidine into water-insoluble nuclear material.(Ref)

The radioactivity increases in proportion to the number of lymphoblasts formed in culture, hence only a simple calculation remain be done.(Ref)
The stimulation index: is the relative increase in 3^H-thymidine incorporation into DNA the the presence of mitogen and antigen compared to the control without the addition of mitogen and antigen. The stimulating index is50-200 for mitogens and over 3 for antigen.

Preparation of substance (A) extracts

For lymphocyte stimulation with Mitogen and antigen lab. test.

- 3.5 gram of substance (A) was finely grinded to a powder.
- In a volumetric buret, 100cc chloroform and then 100cc distilled water were placed. Then substance (A) powder was added and the buret was capped.
- Manual shaking was performed for ½ hour.
- It was hanged in a buret holder overnight.
- Next day the suspension was settled into separate layers inside the buret as shown in the drawing. Each layer is going to be tested separately for it's ability to stimulate lymphocyte culture.

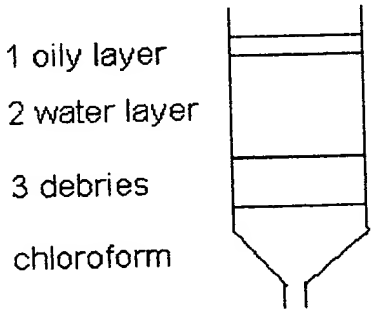

Fig. 1: showing layers formed during extraction of substance A, in the buret hanged overnight.

- Each layer was collected in a different container and kept at 4°C
- The test was performed after 10 days.

Procedure:

1. All cell manipulation was performed using sterile techniques and at room temperature.
2. Blood sample was drawn fresh by venipuncture and anti coagulated with 20 IU heparin /ml from healthy normal females 30-45 years of age.
3. Whole blood was layered gently on 15 ml lymphoprep trying not to disturb the interface then the tube was capped.
4. Centrifuge at 1500 RPM for 30 minutes at 18□C.
5. With a Pasteur pipette and the attached bulb depressed the plasma was discarded. The lymphocyte band removed to be used in the following steps.
6. The lymphocytes was washed with phosphate buffers saline (PBS) and resuspended to a final concentration of 1X10^6 cell concentration /ml in RPMI medium containing penicillin and streptomycin.
7. 0.1 ml of cell suspension was added to *triplicate* wells of microculture plate.

CO_2 incubator.

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1. Antigens and mitogens were added to different plates in triplicates as follows:
   - ◇ PHA mitogen at concentration of 0,50,100,150,200,250 microgram /ml.
   - ◇ PPD antigen at concentration of 0,10,20,30,40,50 microgram /ml.
   - ◇ Substance (A) water extract diluted from stock to ½ , ¼ , 1/8 , 1/16 , 1/32.
   - ◇ Oil extract

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Mean CPM	Normal Control(5) CPM	Normal Control(4) CPM	Normal Control(3) CPM	Normal Control(2) CPM	Normal Control(1) CPM	Conc. Microgm/ml
8395	8290	7799	8558	8438	8888	0
37449	40100	40318	37530	35482	33814	50
46159	49277	54666	45310	44318	46224	100
* 60611	59347	65267	59795	58347	60298	150
53788	51027	55821	55810	54230	52052	200
43126	39400	40500	40324	49722	45686	250

Table(1)

Mean CPM	Patient (5) CPM	Patient (4) CPM	Patient (3) CPM	Patient (2) CPM	Patient (1) CPM	Conc. Microgm/ml
6930	7060	6821	6992	6855	6922	0
33107	32254	36620	34999	31438	30222	50
41440	39274	40210	42371	41786	43558	100
*55749	58002	54029	54102	55332	57279	150
47236	44382	42720	45200	51827	49050	200
41237	38424	39055	44111	43385	41211	250

Table(2)

Dose - response table for mitogen (PHA) stimulation of 10 ^6 lymphocytes

Lymphocytes were pulse – labeled with 2 microcuri of tritrated (H^3) thymidine 20 hours prior to harvesting, counts per minute (CPM) were determined by liquid scintillation spectrometry .

Maximum response occure at 150 microgm/ml

Day	Normal control (1) CPM	Normal control (2) CPM	Normal control (3) CPM	Normal control (4) CPM	Normal control (5) CPM	Mean CPM
0	8104	7999	8220	7550	8040	7983
1	20830	18580	20320	19855	21452	20208
3	60298	58347	59795	65267	59347	60611*
5	36807	35815	37125	32380	30682	34562
7	21892	24722	25340	19005	18772	21946

Table(3)

Day	Patient (1) CPM	Patient (2) CPM	Patient (3) CPM	Patient (4) CPM	Patient (5) CPM	Mean CPM
0	6010	6064	6090	6992	6927	6412
1	17155	16282	17870	20834	16650	17758
3	57279	55332	54102	54029	58002	55749*
5	33125	36222	34672	30604	30110	32947
7	20621	18046	19696	16500	18644	18741

Table(4)

Time - response table for mitogen stimulation (PHA) or 10^6 Lymphocytes.

Peripheral blood lymphocytes using an with an optimal concentration of (PHA) (150 microgm/ml).

Maximal response occured at (3) days after initiating .

Conc. Microgm/ml Of PPD	Control(1) CPM	Control(2) CPM	Control(3) CPM	Control(4) CPM	Control(5) CPM	Mean CPM
0	8044	7915	7866	8022	7890	7947
10	14189	13885	13262	14552	13830	13944

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20	26063	24660	24322	23380	26608	25007
30	38115	36239	36811	38404	43750	36864*
40	28362	25466	25444	28755	27358	27077
50	20069	19920	20508	22210	19250	20391

Table(5)

Conc. Microgm/ml PPD	Patient (1) CPM	Patient (2) CPM	Patient (3) CPM	Patient (4) CPM	Patient (5) CPM	Mean CPM
0	7814	7855	8020	7944	7828	7892
10	13208	12332	13642	13928	12686	13159
20	25882	24246	23676	23782	26993	24916
30	36446	34313	35267	35320	34875	35244*
40	27351	28360	25984	28262	26989	27389
50	20485	20230	19590	21623	18125	20011

Table(6)

Dose-response table for antigen stimulation (PPD) at 10^6 lymphocytes.

Culture were harvested for 120 hours at culture after 20 hours with tritiated (H^3) thymidine counts per minut (CPM) were determined by liquid scintllation spectrometry.

maximum response is at 30 microgm/ml of antigen.

Day	Control(1) CPM	Control(2) CPM	Control(3) CPM	Control(4) CPM	Control(5) CPM	Mean CPM
0	8000	7774	8022	8004	7908	7942
1	14351	12556	13814	12500	13212	13287
3	26682	22111	26823	20251	24815	24267
5	38115	36239	36811	38404	34750	36864*
7	25166	23750	29402	28987	26223	26666
9	19758	21482	22650	20829	18860	20716

Table(7)

Day	Patient (1) CPM	Patient (2) CPM	Patient (3) CPM	Patient (4) CPM	Patient (5) CPM	Mean CPM
0	7914	8366	7390	7801	7729	7840
1	13828	11680	13362	13318	12892	13016
3	22416	21120	22444	19755	22365	21620
5	36466	34313	35267	35320	34875	35248*
7	26069	24920	25508	27538	27820	26371
9	15622	19414	20282	19620	19351	18858

Table(8)

Time-response table for antigen stimulation (PPD) of 10^6 lymphocytes.

Antigen concentration for all cultures was optimal concentration (30 microgm/ml).

Maximum response occurred on day (5) of culture .

Dilution of water extrac.	Control(1) CPM	Control(2) CPM	Control(3) CPM	Control(4) CPM	Control(5) CPM	Mean CPM
0	7914	8366	7210	7001	7724	6743
1/32	13828	11680	13362	13318	12892	13016
1/16	22416	21120	22444	19755	22365	21620

1/8	36466	34313	35267	35320	34875	35248 *
1/4	26099	24920	25508	27538	27820	26377
1/2	15622	19414	20282	19620	19351	18858

Table I

WATER EXTRACTION

Dilution of oil	Control(1) CPM	Control(2) CPM	Control(3) CPM	Control(4) CPM	Control(5) CPM	Mean CPM
0	7914	8366	7210	7001	7724	7643
1/32	13984	14282	11989	12485	14230	13394
1/16	24590	22623	26125	22450	24687	24096
1/8	35897	33389	34391	35302	33155	34427*
1/4	26260	26118	26620	27827	25033	26372
1/2	19250	21623	19357	20820	16755	19621

Table II

OIL EXTRACTION

Dilution of (x)	Control(1) CPM	Control(2) CPM	Control(3) CPM	Control(4) CPM	Control(5) CPM	Mean CPM
0	7914	8366	7210	7001	7724	7643
1/32	12355	11382	13141	10500	12382	11952
1/16	24026	22630	21435	20702	22350	2229
1/8	34153	33455	33897	35389	35380	34455*
1/4	24977	22750	22370	23268	24662	23605
1/2	17275	18320	19779	19233	20631	19048

error

Table III

(X) EXTRACTION

Dose response table for substance a stimulation of 10^6 lymphocytes
Maximum response occures at (1/8) dilution of the stock extract.

Day	Control(1) CPM	Control(2) CPM	Control(3) CPM	Control(4) CPM	Control(5) CPM	Mean CPM
0	8000	7774	8022	7908	8004	7942
1	13208	12332	13642	13928	12686	13159
3	25882	24246	23676	23782	26993	24916

5	36446	34313	25267	35320	34875	35244
7	27351	28360	25984	28262	26989	27389
9	20485	20230	19590	21623	18125	20011

Table IV

WATER EXTRACTION

Day	Control(1) CPM	Control(2) CPM	Control(3) CPM	Control(4) CPM	Control(5) CPM	Mean CPM
0	8000	7774	8022	7908	8004	7942
1	14354	12351	12225	12894	11262	12617
3	20311	21230	24709	20174	23248	21934
5	35897	33389	34391	35302	33155	34427
7	24211	21150	23830	25844	25056	24018
9	16617	18250	19262	17692	18450	18054

Table V

OIL EXTRACTION

Day	Control(1) CPM	Control(2) CPM	Control(3) CPM	Control(4) CPM	Control(5) CPM	Mean CPM
0	8000	7774	8022	7908	8004	7942
1	11004	12131	12222	11623	12357	11867
3	20820	19755	23355	20382	22141	21291
5	34153	33455	33897	35389	35380	34455
7	25628	24265	25632	28623	26122	26054
9	16253	18048	19225	19220	17852	18120

Table III

CHLOROFORM EXTRACT

Dose response table for substance astimulation of 10^6 lymphocytes .
Maximum response occures at (1/8) dilution of the stock extract.

CLAIMS DESCRIPTION OF THE INVENTION

A NEW ASTHMA THERAPY THAT ACT ON EOSINOPHILS AND/OR T LYMPHOCYTES

① NOVEL USE OF A NATURAL HERB NIGELLA SATIVUM IN PREPARIN CAPSULES OR SUSPENSION FOR ASTHMA AN ALLERGY TREATMENT IN HUMANS + OTHER INDICATONS (Page 2)

T- LYMPHOCYTE STIMULATION TEST SHOWED THAT NIGELLA IS A T- LYMPHOCYTE STIMULANT. IT IS A NOVEL IMMUNOMODULATOR.

② THE USE OF GLICOFOSFOPEPTICAL IN PREPARING AN ASTHMA AND ALLERGY DRUG USING IT IN A NOVEL SCHEDULE OF THERAPY OF 5-20 DAY ONLY (FIVE — TWENTY days only) (AN IMMUNOMODULATOR WHICH IS MARKETED)

③ THE SHORT TERM USE OF ALL IMMUNOMODULATORS IN ASTHMA

Measurements of Lymphocyte activation and proliferation after stimulating them by substance (A) extracts, comparing it to know Antigen and Mitogen.

Abstract:

Measurement of lymphocyte activation and proliferation is an invitro technique for the measurement of cell mediated immunity.

The proliferatine capacity of lymphocytes is measured by their ability to incorporate tritiated thymidine, the newly synthesized DNA in culture. The radioactive is measured by liquid scintillation spectrometer various Mitogens, Antigens, Cytokines or Antibodies are used as a stimulant .

In this study purifide protein Derivitine (PPD), Phytohaemagglutination (PHA) and substance (A) extracts were used as stimulants.

A dose response test to determine the optimal dose for stimulation of this batch lymphocytes .then this optimal dose is used in a time response to detect the time need for maximal incorporation of H3 thymidine .

Results of this study had shown similarity of oil, water, and chloroform extracts of substance A with PPD.

Introduction:

Lymphocyte stimulation is an in vitro technique that commonly is used to assess cellular immunity in cancer, infectious diseases, immunodeficiency and autoimmunity. It was first reported by by Nowel in 1966*. Lymphocytes are stimulated in vitro to become metabolically active by antigens (by antigen) or mitogens. Cell division results in increased DNA synthesis, and 3H-thymidine incorporation (REF. 1) often is used as an indicator of that synthesis.

The ability of substance (A) to activate lymphocytes in culture was studied. Extracts proliferation and lymphocytes transformation is measured by the incorporation of tritiated thymidine in the newly synthesized DNA. The radioactivity increases in proportion to the number of lymphoblasts and can be measured by liquid scintillation spectrometry. Results showed that water, oil and X extracts from substance (A) has got a stimulatory effect on lymphocytes in culture. Similar to PPD, which is an Antigen that can be used for immunotherapy in humans.

This technique consists of placing cultured lymphocytes *in-vitro* in contract with a known concentration of mitogen, or antigen to which they might be sensitive. If the reaction is positive, the lymphocytes is transformed into lymphoblast (6).

An in vitro correlate of cell mediated immunity (CMI).

Is a determination of T-cell prolifermination.** This method evaluates the capacity of T-lymphocytes that have been primed in vivo to respond in vitro after culture with the appropriate antigen.

The first step in this response is the interaction of antigen specific T-cells with antigen-presenting cells. After recognition of the specific antigen, the T-cell undergoes a series of physiologic changes resulting in its transformation to a lymphoblast and culminating in cell division.